HEAT RESISTANCE STUDIES ON CLOSTRIDIUM BOTULINUM IN MEAT

BY

CHESTER WARREN HOUSTON

B. S., University of Illinois, 1939

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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY
IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1947

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HEAT RESISTANCE STUDIES ON CLOSTRIDIUM BOTULINUM IN MEAT

The literature is profuse with reports of thermal resistance of microorganisms, especially those which are involved in the spoilage of canned foods. Of these, the spore-forming anaerobic organisms are extremely heat resistant, and many studies have been made on the thermal death times of the spores of these organisms. It has long been known that the menstruum in which spores are heated is an important factor in heat resistance studies; yet studies of these organisms suspended in meat are few. Consequently, it was decided to study the resistance of *Clostridium botulinum* suspended in a meat substrate and to determine from the collected data the thermal death time curve. *Clostridium botulinum* was selected as the test organism since data for allied anaerobes are available and, due to this organism's wide distribution, it might be present in meat.

The procedure used in this study was essentially that of Stumbo, Gross, and Vinton (1945). Spores of *Clostridium botulinum*, a type A and a type B strain (American Type Culture Collection Numbers 7948 and 7949, respectively), were grown in four different media and suspended in meat in three concentrations, namely, 100, 1000, and 10,000 spores per gram of meat. The meat used was the lean trimmed from the standing ribs of grade A beef, and upon analysis was found to have a fat and moisture content of 10% and 68%, respectively. The pH was 6.7.

The media used for the production of spores were the liver broth of Cameron, Williams, and Thompson (1928), brain peptone medium of Hall (1920), glucose veal medium of Nevin (1921), and the brain medium of Stumbo, Gross, and Vinton (1945). Cultures for spore production were incubated for 8 days at 37°C., at the end of which time they were filtered through cheese cloth and cotton, given a spore

test, and stored in the refrigerator at 4°C. The spores were approximately four months old when the determinations reported were run. A suitable quantity of the stock suspension, or a dilution thereof, was added to a weighed quantity of ground lean beef. The spores were distributed in the meat by means of a Stevens electric mixer. The meat was dispensed in 10 × 75 millimeter Kimble glass culture tubes. After sealing, the tubes were stored for 24 hours at 4°C., at the end of which time they were removed, pre-heated for five minutes at 79.4°C. and heated for various times at the desired temperatures in a constant temperature oil bath. Five tubes were run at each processing level. Tests for survival were carried out in the sealed tubes. Survival of the test organism was evidenced by darkening and digestion of the meat, and by gas production. Microscopic examination was made for typical morphology, and toxicity tests were run on occasional positive tubes for confirmation of toxin production.

Thermal death time curves are presented for spores of *Clostridium botulinum* produced in the various media and suspended in ground lean beef in concentrations of 100, 1000, and 10,000 spores per gram of meat. Curves which were drawn after the plotting of all end points from all determinations of any one concentration on the same graph paper are also given.

The resistance of the spores grown in the various media differed somewhat, as did the spores of the two strains. The variations were not considered great enough to be of significance.

From the curves presented, the "F" and "z" values, which are the pertinent figures as regards process calculations for canned foods, were determined. These values are defined as follows:

F = the number of minutes required to destroy the organism in question at 250°F.

z = the number of degrees Fahrenheit over which the

curve passes in traversing one logarithmic cycle, a value which represents the slope of the thermal death time curve.

Values of "z" obtained from the curves drawn for the individual determinations varied from 13.0 to 22.0; however, the majority were in the range of 17 to 19. From the composite plottings of all determinations using any one concentration, "z" values ranged from 16 to 18. These curves are considered more reliable than the ones obtained from the individual determinations, and the "z" values obtained therefrom are comparable to those reported by Townsend, Esty, and Baselt (1938) for *Clostridium botulinum* in neutral phosphate. Within the limits of the concentrations used, it appears that the concentration of spores per gram of meat has little effect on the slope of the thermal death time curve, the "z" value.

Values of "F" from the curves drawn over the composite plottings were 0.24, 0.46, and 0.75 for 100, 1000, and 10,000 spores per gram of meat, respectively. An increase in the time necessary for destruction at various temperatures with increasing concentration of spores was observed; this fact was also shown by the increasing values of "F."

Townsend, Esty, and Baselt (1938) reported that the slope of the thermal death time curve of *Clostridium botulinum* was lower in food and food juices than the slope of the curve determined for the organism in neutral phosphate. This fact was not observed for the two strains studied in this investigation when the spores were suspended in ground lean beef.

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VITA

The author was born March 5, 1918, in Champaign County, Illinois, where he attended Equity Elementary School and Champaign High School, graduating from the latter in 1935. The Bachelor of Science and Master of Science Degrees were conferred by the University of Illinois in 1939 and 1940, respectively. During the summer of 1939, he was employed by the Minnesota Valley Canning Company, LeSueur, Minnesota. From 1940 to 1942, he was employed in the Research Department of the Continental Can Company, Chicago, Illinois. For the following three years, he served in the United States Navy. He returned to the University of Illinois in October, 1945, and was a teaching assistant in the Department of Bacteriology for two semesters. He is a member of Sigma Xi, Phi Sigma, and the Society of American Bacteriologists.

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